

CLAIMS

1. A neutral phenol oxidase having the following properties:

(1) optimum pH: being from 5.0 to 7.0, and

5 (2) substrate specificity:

i) catalyzing a coloring reaction by an oxidation of each of N,N-dimethyl-para-phenylenediamine, ortho-aminophenol, 2,6-dimethoxyphenol, 1,3-dihydroxynaphthol, and 4-hydroxyindole at a pH of around 6.5; and

ii) catalyzing an oxidative polymerization reaction of alkali extract of lignin.

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2. The neutral phenol oxidase according to claim 1, further having the following properties (3) to (7):

(3) 28 kDa (calculated by SDS-PAGE),

(4) pH stability: maintaining a relative remaining activity of at least 70 % under incubation conditions at a pH of 8.0 to 9.0 for 20 hours at 30°C,

15 (5) optimum temperature: being from 30° to 50°C,

(6) thermal stability:

I) maintaining a relative remaining activity of at least 80 % under incubation conditions at 0° to 30°C for 1 hour at a pH of 7.0, as compared with an activity before 20 incubation, and

II) maintaining a relative remaining activity of at least 90 % under incubation conditions at 0 to 30°C for 1 hour at a pH of 9.0, as compared with an activity before incubation, and

(7) isoelectric point: being about 7.4;

25 or the following properties (3') to (7'):

(3') 35 kDa (calculated by SDS-PAGE),

(4') pH stability: maintaining a relative remaining activity of at least 75 % under incubation conditions at a pH of 7.0 to 10.0 for 20 hours at 30°C,

(5') optimum temperature: being from 30° to 60°C,

5 (6') thermal stability:

I') maintaining a relative remaining activity of at least 90 % under incubation conditions at 0° to 50°C for 1 hour at pH 7.0, as compared with an activity before incubation, and

10 II') maintaining a relative remaining activity of at least 70 % under incubation conditions at 0° to 50°C for 1 hour at a pH of 9.0, as compared with an activity before incubation, and

(7') isoelectric point: being about 6.8;

or the following properties (3") to (7"):

(3") 45 kDa (calculated by SDS-PAGE),

15 (4") pH stability: maintaining a relative remaining activity of at least 70 % under incubation conditions at a pH of 8.0 to 10.0 for 20 hours at 30°C,

(5") optimum temperature: being from 30° to 60°C

(6") thermal stability:

20 I") maintaining a relative remaining activity of at least 80 % under incubation conditions at 0° to 30°C for 1 hour at a pH of 7.0, as compared with an activity before incubation, and

II") maintaining a relative remaining activity of at least 90 % under incubation conditions at 0° to 40°C for 1 hour at pH 9.0 , as compared with an activity before incubation, and

25 (7") isoelectric point: being about 6.8.

3. The neutral phenol oxidase according to claim 1 or 2, wherein the neutral phenol oxidase is produced by a basidiomycete belonging to the genus *Flammulina*.

5 4. The neutral phenol oxidase according to any one of claims 1 to 3, wherein the basidiomycete belonging to the genus *Flammulina* is a basidiomycete belonging to *Flammulina velutipes*.

10 5. The neutral phenol oxidase according to any one of claims 1 to 4, wherein the basidiomycete belonging to *Flammulina velutipes* is *Flammulina velutipes* IFO 30601 strain.

15 6. A production method of a neutral phenol oxidase, characterized in that a basidiomycete belonging to the genus *Flammulina* is cultured at a pH of 6.0 to 12.0.

7. The production method according to claim 6, wherein the neutral phenol oxidase is the neutral phenol oxidase of claim 1 or 2.

8. An antibody raised against the neutral phenol oxidase of any one of claims 1 to 5.

20 9. A dyeing composition comprising the neutral phenol oxidase of any one of claims 1 to 5.

10. The dyeing composition according to claim 9, further comprising a dye.

11. A dyeing method, characterized in that a subject to be dyed is brought into contact with a dye in the presence of the neutral phenol oxidase of any one of claims 1 to 5, thereby dyeing the subject to be dyed.